IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Habib Zaghouani, et al.	ATTORNEY DOCKET:	0119742.00002
SERIAL NO.:	10/510,411	GROUP ART UNIT:	1644
FILED:	September 12, 2005	EXAMINER:	Ewoldt, Gerald R.
TITLE:	Methods and compositions for treatment, prevention, suppression and/or delaying the onset of type 1 diabetes		

DECLARATION UNDER 37 C.F.R. § 1.132

I, Habib Zaghouani, do hereby declare and say:

1. I am a citizen of the United States and my current residential address is 1608 Brookfield Manor, Columbia, Missouri, 65203.

2. I obtained my undergraduate degree in biochemistry from University of Paris, France in 1981. I obtained a Ph.D. in immunology from the University of Paris/Cancer Research Institute, France in 1987.

3. I am presently the J. Lavenia Edwards Chair in Pediatrics, Director, Center for Cellular and Molecular Immunology and Professor, Department of Molecular Microbiology & Immunology and Department of Child Health at the University of Missouri.

4. I have over one hundred publications and abstracts in the field of immunology. Please refer to the copy of my *curriculum vitae* in attached Appendix A for more details.

5. I am a named inventor on the '411 application as well as on related co-pending application serial numbers: 10/681,788; 11/290,070; and 11/425,084.

6. I have performed experiments examining the impact of administration, initiated at the pre-diabetic stage, of soluble Ig-GAD2 to NOD mice over a period of 56 weeks. Data are provided in attached Appendix B.

7. NOD mice were assessed for blood glucose beginning at week 12 of age. Those mice

that reached glucose levels of 160 – 250 mg/dl between week 14 to 25 received the following Ig-GAD2 regimen: 500 μg of soluble Ig-GAD2 i.p. daily for 5 days and then weekly injections thereafter for either 15 or 25 weeks. Blood glucose monitoring was performed during this period.

8. Overall, 100% of mice that became pre-diabetic at the age of 14 – 25 weeks and that were not treated with Ig-GAD2 progressed to diabetes (blood sugar level ≥300 mg/dl glucose) within 5 weeks after diagnosis of the pre-diabetic stage. Moreover, 60% of mice undergoing the 15-week treatment regimen were protected against diabetes throughout the 25 week post-hyperglycemia monitoring period. Interestingly, one mouse (Figure 1 B, left panel, open stars) progressed to diabetes by 5 weeks of treatment and 3 mice (Figure 1 B, plus, open diamond, and open pentagon) had similar disease manifestations shortly after interruption of the treatment.

9. When the regimen was extended to 25 weeks, 100% of the Ig-GAD2 treated animals were protected (Figure 1 A, right panel) and normoglycemia was restored in all mice (Figure 1 B, right panel). This status persisted throughout the duration of the study, which was terminated when the mice were 52 to 56 weeks of age.

10. Detailed histopathologic analysis from the mice was performed. While most of the islets in hyperglycemic and diabetic control mice exhibited intrainsulitis (Figure 2, panels 1, 2 and 3), the majority of islets in treated mice were not inflamed (Figure 2, panel 4) or had only mild periinsulitis (Figure 2, panels 5 and 6).

11. Overall, the histopathologic analysis indicated that treated mice had significantly greater number of islets when compared to both hyperglycemic and diabetic mice (Figure 3). The number of insulin-positive islets also increased from 14 per pancreas at the prediabetic stage to 29 per pancreas upon treatment with soluble Ig-GAD2. Analysis of islet infiltration scores among the different groups of mice indicated that the 15-week treatment group had a higher number of islets with periinsulitis (38% vs. 30%) or no insulitis (35% vs. 17%) relative to the hyperglycemic stage (Figure 4). On the other hand, the number of islets with severe- and mild-intrainsulitis were reduced in the treated versus hyperglycemic mice (8% and 19% vs. 22% and 31%, respectively) (Figure 4).

12. Surprisingly, in the 25-week treatment group, although the total number of islets was reduced to that of the hyperglycemic stage, most of these islets exhibited no (60%), peri- (28%) or mild intra- (12%) insulitis (Figure 4). Overall, the treatment with Ig-GAD2 led to a significant increase in the number of noninflammed ("healthy") islets that restored and maintained normoglycemia.

13. An experiment was performed to determine whether the healthy islets discussed above were a result of a regression of inflammation and/or regeneration of beta cells. To address this question, the treated mice were injected with the proliferation indicator 5-bromo-2'-deoxyuridine (BrdU), sacrificed and pancreatic sections were stained with anti-insulin and anti-BrdU antibodies and analyzed for BrdU incorporation and insulin production.

14. BrdU staining was visible in the highly proliferative luminal intestinal cells but these had no staining with anti-insulin antibody (Figure 5). Islets of non-diabetic 5-week old NOD mice were positive when stained with anti-insulin antibody, but did not incorporate BrdU, suggesting that these insulin-producing beta cells were not newly generated cells (Figure 6). Thus, under normal circumstances insulin production emanates from existing beta cells whose nuclei do not incorporate BrdU giving a minimal number of BrdU/insulin double-positive ($BrdU^+/insulin^+$) beta cells (Figure 7). Sections from hyperglycemic mice showed very few insulin-producing beta cells and no BrdU incorporation (Figure 8) resulting in an insignificant number of $BrdU^+/insulin^+$ beta cells (Figure 7). In contrast, islets from the 25-week treatment group showed beta cells that stained positive for insulin and were either BrdU negative (previously generated beta cells) or BrdU positive (newly generated beta cells) (Figure 9). Notably, the number of these insulin-producing regenerating beta cells was significantly increased in all five mice in which treatment restored normoglycemia (Figure 7).

15. Interestingly the total number of regenerating cells producing insulin ($Insulin^+/BrdU^+$) was low and may not solely account for the restoration of normoglycemia. Insulin-positive / BrdU-negative islet cells may have also contributed to the control of blood glucose level and these likely represent a combination of newly regenerated and

previously existing beta cells. There were also numerous BrdU positive / insulin-negative islet cells that likely represent newly regenerating cells that are not yet producing abundant insulin (Figure 9).

16. Splenic cells from recovered mice were then stimulated with GAD2 peptide and assessed for both suppressive and inflammatory cytokines. The results indicated that although no measurable IL-4 or TGFβ was detected (not depicted), there was significant IFNγ and IL-10 production by these cells relative to the control HEL peptide (Figure 10).

17. When *in vivo* cytokine neutralization was performed along with soluble Ig-GAD2 treatment, the recovery persisted with anti-IL 10 treatment but was nullified by removal of IFNγ (Figure 11). These results indicate that IFNγ (but not IL-10), contrary to IFNγ's well-defined inflammatory function, is likely involved in modulation of inflammation and restoration of normoglycemia.

18. Th17 cells represent a newly defined subset of pathogenic T cells whose development can be facilitated by TGFβ and IL-6 or interfered with by IFNγ or IL-27. Because soluble Ig-GAD2 treatment induces IFNγ, we sought to determine whether restoration of normoglycemia involves interference with IL-17 production. As such, an experiment was performed to assess whether IL-17 is produced by NOD T cells and, if so, to follow its pattern of secretion during disease progression.

19. IL-17 was evident upon IAA-seroconversion and increased significantly when the mice progressed to hyperglycemia and diabetes. Treatment with soluble Ig-GAD2 at the hyperglycemic stage significantly reduced the frequency of GAD2-specific IL-17 producing cells as measured by spot formation (data not shown). However, neutralization of IFNγ by administration of anti-IFNγ antibody along with Ig-GAD2 restored even higher frequency of Th17 cells which was likely caused by complete neutralization of IFNγ (data not shown). It is therefore likely that the restoration of diabetes by neutralization of IFNγ during treatment with Ig-GAD2 as seen in Figure 11 is actually caused by restoration of Th17.

20. At the time of filing of the '411 application T1D was suspected to involve multiple

autoantigens and the initiating antigen was unknown. As such, a person of ordinary skill in the art reading the literature at that time would have expected that induction of tolerance, if even possible, would require modulation of diverse T cell specificities through a combination of peripheral tolerance and bystander suppression.

21. It was known prior to filing of the '411 application that cross-linking of Fc receptors on target cells by antigen-antibody complexes could trigger the production of cytokines such as IL-10 which are important for down regulating T cells engaged to antigen presenting cells as well as neighboring T cells (*e.g.* bystander suppression).

22. Additionally, at the time of the '411 application, aggregation of Igs was known to confer effector functions associated with the Fc fragment without the need for complex formation.

23. In view of the foregoing, at the time the '411 application was filed, a person of ordinary skill in the art would not have believed that a ***soluble*** Ig-peptide chimera would be effective at treating T1D because such a person would not have expected a soluble Ig chimera to cross-link Fc receptors and stimulated cytokines (*e.g.* IL-10) by APCs so as to stimulate bystander suppression.

24. Surprisingly, we have found that soluble Ig-GAD2, despite the fact that it does not induce cross-linking of Fc receptors, delayed T1D when administered at the preinsulitis stage and reversed diabetes when given at the prediabetic stage whereas aggregated Ig-GAD2 (which would be expected to cross-link Fc receptors and induce IL-10 secretion by APCs) did not delay diabetes when given after IAA seroconversion (unpublished data).

25. In my opinion, at the time of filing of the present application a person of ordinary skill in the art would not have expected that the presently claimed soluble Ig-GAD2 construct but not the aggregated Ig-GAD2 construct would delay T1D when administered after IAA seroconversion—a relatively advanced stage of disease.

26. In my opinion, a person of ordinary skill in the art, at the time of filing the present application, would not have expected that an antigen-specific single-epitope therapy such as soluble Ig-GAD2 would restore normoglycemia in hyperglycemic NOD mice.

27. It would have been particularly surprising to the person of ordinary skill in that art that soluble Ig-GAD2 was able to restore normoglycemia through an IL-10-independent mechanism. Such a person would certainly not have predicted that soluble Ig-GAD2 would delay T1D and reverse disease in pre-diabetic mice by inducing IFNγ to suppress IL-17 which is contrary to IFNγ's well known inflammatory function.

28. Moreover, in my opinion, at the time of filing the present application a person of ordinary skill in the art would not have expected that treatment with the claimed soluble Ig-GAD2 construct would lead to an increase in the number of healthy, insulin positive pancreatic islet cells in NOD mice by comparison with controls.

29. I declare that all statements made herein of my own knowledge and are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

05/04/2009
Date

Habib Zaghouani, PhD

APPENDIX A

Curriculum Vitae Habib Zaghouani

EDUCATION

Ph.D.	1987	Immunology, University of Paris/Cancer Research Institute, Paris, France.
M.S.	1983	Immunology, University of Paris/Pasteur Institute, Paris, France.
B.S.	1981	Biochemistry, University of Paris, Paris, France.

POSITIONS AND RESEARCH EXPERIENCE

2006-present	Director, Center for Cellular and Molecular Immunology, The University of Missouri School of Medicine, Columbia, MO
2006-present:	J. Lavenia Edwards Chair in Pediatrics, the University of Missouri School of Medicine, Columbia, MO
2006-present:	Professor, Department of Child Health, the University of Missouri School of Medicine, Columbia, MO.
2001-present:	Professor, Department of Molecular Microbiology and Immunology, the University of Missouri School of Medicine, Columbia, MO.
2000-2001:	Associate Professor, Department of Microbiology, the University of Tennessee, Knoxville, Tennessee.
1994-2000:	Assistant Professor, Department of Microbiology, the University of Tennessee, Knoxville, Tennessee.
1990-1994:	Research Assistant Professor, Department of Microbiology, Mount Sinai School of Medicine, New York.
1987-1989:	Postdoctoral Fellow, Department of Microbiology, Mount Sinai School of Medicine, New York. Mentor: Dr. Constantin A. Bona.
1983-1987:	Graduate Research Assistant, Ph.D. candidate, Immunology, University of Paris/Cancer Research Institute, Paris, France. Mentor: Dr. Marc Stanislawski.
1981-1983:	Graduate Research Assistant, M.S. candidate, Immunology, Pasteur Institute, Paris, France. Director: Dr. Arthur Dony Strosberg.

RESEARCH GRANT SUPPORT

A. Principal Investigator

Active

1). **2RO1 NS 037406**, National Institutes of Health, March 2004 - February 2009. Modulation of autoreactive T cells. PI: Habib Zaghouani.

2). **1RO1 DK 065748**, National Institutes of Health, April 2005-March 2008. Immune tolerance against type I diabetes in mice. PI: Habib Zaghouani.

3). **2RO1 AI 48541**, National Institutes of Health, May 2006- April 2011. Regulation of neonatal immunity. PI: Habib Zaghouani.

4). **1R21 AI 068746**, National Institutes of Health. July 2007 – June 2009. Mimotopes against type I diabetes. PI: Habib Zaghouani.

Pending

1). **1RO1 NS057194-A2**, National Institutes of Health, April 2008 - March 2013. Regulation of autoimmune encephalomylitis. PI: Habib Zaghouani. **8.7 percentile**
(a)

2). **2RO1 DK 065748-01**, National Institutes of Health, April 2008-March 2013. Immune tolerance against type I diabetes in mice. PI: Habib Zaghouani. **35 percentile**

B. Co-investigator, Mentor, or Key Personnel

Active

T32 GM008396, National Institute of General Medical Sciences (NIGMS), July 1991-June 2012. Molecular Basis of Gene Expression and Signal Processing. PI: Mark Hannink (Zaghouani: Mentor).

T32 RR007004, National Institutes of Health, July 2005-June 2010, Postdoctoral Training in Comparative Medicine. PI: Craig Franklin (Zaghouani: Mentor).

T90 DK71510, National Institutes of Health, September 2004 – August 2009. Bench and Back: Clinical biodetectives training. PI: Mark Milanick (Zaghouani: Mentor).

R90 DK71510, National Institutes of Health, September 2004 – August 2009. Bench and Back: Clinical biodetectives training. PI: Mark Milanick (Zaghouani: Mentor).

KO8 AR048671, National Institutes of Health, June 2005-April 2008, Cytokine regulation of collagen-induced arthritis. PI: Robert Ortman (Zaghouani: Mentor).

1G20 RR021327, National Institutes of Health, September 2004-August 2009. Equipment for the MU Life Sciences Center. PI: Lon Dixon, (Zaghouani: Key personnel).

1 G20 RR019711, National Institutes of Health, September 2004-Agust 2009. Renovation of MU Medical School Vivarium. PI: Lon Dixon. (Zaghouani: Key personnel).

U19AT003264-01, National Institutes of Health, September 2005 – August 2009. TICIPS: HIV/AIDS, Secondary Infections and Immune Modulation. Center grant. PI: William Folk (Zaghouani: Faculty Member).

Research Foundation Grant, Arthritis Foundation, April 2006 – May 2008. Synoviolin is a target for arthritis. PI: Deyu Fang (Zaghouani: Mentor).

C. Previous Support (PI: Zaghouani, H)

1). R21 AI 062796, National Institutes of Health, July 2005-June 2007. Immune tolerance in the newborn mouse. Yearly direct cost $150,000. PI: Habib Zaghouani. No cost extension 11/30/2007

2). 1RO1 AI48541, National Institutes of Health, May 2001- April 2006. Regulation of neonatal immunity. Yearly direct cost: $175,000. PI: Habib Zaghouani.

3). Astral Inc, October 2001- September 2004. Development of Approaches to Combat Autoimmunity. PI: Habib Zaghouani.

4). RO1NS37406, National Institutes of Health, January 2000- December 2004. Modulation of autoreactive T cells. PI: Habib Zaghouani

5). RG2967B-3, National Multiple Sclerosis Society, October 2002 – March 31, 2004 Down-regulation of encephalitogenic T cells. PI: Habib Zaghouani.

6). RG2967A2/1, National Multiple Sclerosis Society, April 99 - March 2002. Down-regulation of encephalitogenic T cells. PI: Habib Zaghouani.

7). Astral Inc: March 95 - July 2001. A novel approach to delete encephalitogenic T cells. PI: Habib Zaghouani.

8). RG2778A1/1, National Multiple Sclerosis, April 96 - March 1999. A deletional strategy for encephalitogenic T cells. PI: Habib Zaghouani.

9). Astral Inc:, September 97- August 99. Generation of human Ig chimeras carrying wild type or antagonist forms of myelin peptides. PI: Habib Zaghouani.

10). 1R41AI47496, (STTR): National Institutes of Health, September 2000-August 2001. Treatment of EAE using a novel delivery system. . Co-PI: Habib Zaghouani.

TEACHING EXPERIENCE

2004:	Microbiology 205 (Medical Microbiology) 3 credit hours, 8 lecture contact hours, 170 student, Spring semester, University of Missouri School of Medicine, Columbia.
2002-present:	Microbiology 304 (Immunology) 3 credit hours, 14 lecture contact hours, 30 students, Fall semester, Molecular Microbiology and Immunology, University of Missouri School of Medicine, Columbia.
2002-present	Microbiology 407 (advanced Immunology) 4 credit hours, 9 lecture contact hours, 18 students, Spring semester, Molecular Microbiology and Immunology, University of Missouri School of Medicine, Columbia.
2001-present:	Bio 4952, Undergraduate research, 3 credit hours, 1-2 students, Fall and Winter semesters
2001-present:	Bio 4950, Undergraduate research, 3 credit hours, 2-3 Students, Fall and Winter semesters
2001-present:	Direct Immunology Journal Club, 1hour/week all year around, 40 student, postdocs and faculty members
1995-2001:	Microbiology 430 (Immunology), 3 credit hours, 45 lecture contact hours, 100-120 students, Fall semester, Microbiology, The University of Tennessee, Knoxville.
1995-2001:	Co-direct Microbiology 602 (Microbial Pathogenesis Journal Club), 1 credit hour, 15 lecture contact hours, 10-15 students, Fall semester, Microbiology, The University of Tennessee, Knoxville.
1995-2001:	Co-direct Microbiology 603 (Immunology Journal Club), 1 credit hours, 15 lecture contact hours, 10-15 students, Spring semester, Microbiology, The University of Tennessee, Knoxville.
1995-2001:	Microbiology 401 (Undergraduate Research), 3 credit hours, 1-2 students per semester, Microbiology, The University of Tennessee, Knoxville.

1998: Microbiology 630 (Topics in Immunology), 3 credit hours, 10 lecture contact hours, 20 students, Spring semester, (Seminar Series) Microbiology, The University of Tennessee, Knoxville.

1998-2001: Microbiology 493 (Independent Study in Immunology), 6 students, 10 lecture contact hours, spring, Microbiology, The University of Tennessee, Knoxville.

1992-1994: 600-level Immunology course, 3 credit hours, 6 lecture contact hours, 10 students, spring, Microbiology, Mount Sinai School of Medicine, New York.

HONORS AND AWARDS

2006. Speaker, Keystone Symposia on Tolerance Autoimmunity and Immune Regulation. March 21-26, 2006. Beaver Run Resort, Breckenridge, Colorado. Presentation title: Tregs for or against diabetes.

2004: Research Equipment Award for the purchase of an ELISPOT Analyzer, Office of Research, The University of Missouri,

2003: Keystone Symposia Scholarship ($1,000) for poster presentation by Hyun-Hee Lee, a graduate student in the laboratory, the meeting was held in Snowbird, UT

2003: Honorable citation for poster presentation by Randal Gregg, a graduate student in the laboratory. Life Science week, University of Missouri-Columbia.

2001: Science Alliance Research Excellence Award, Oak Ridge National Laboratories and The University of Tennessee, Knoxville.

2000: Science Alliance Research Excellence Award, Oak Ridge National Laboratories and The University of Tennessee, Knoxville.

2000: Exhibit, Performance, and Publication Expense Award, Faculty Senate Research Council and Office of research Administration, The University of Tennessee, Knoxville.

1999: Chancellor's nomination for Howard Hughes Medical Institute Assistant Investigator Appointment, The University of Tennessee, Knoxville.

1999: Biological Equipment Award, Office of Research Administration/Science Alliance/Genome Science and Technology/Division of Biology, The University of Tennessee, Knoxville.

1999: Science Alliance Research Excellence Award, Oak Ridge National Laboratories and The University of Tennessee, Knoxville.

1999: Exhibit, Performance, and Publication Expense Award, Faculty Senate Research Council and Office of research Administration, The University of Tennessee, Knoxville.

1998: Science Alliance Research Excellence Award, Oak Ridge National Laboratories and The University of Tennessee, Knoxville.

1998: Exhibit, Performance, and Publication Expense Award, Faculty Senate Research Council and Office of Research Administration, The University of Tennessee, Knoxville.

1997: Biological Equipment Award, Office of Research Administration/Science Alliance/ Division of Biology/ Department of Microbiology, The University of Tennessee, Knoxville.

1997: Exhibit, Performance, and Publication Expense Award, Faculty Senate Research Council and Office of Research Administration, The University of Tennessee, Knoxville.

1990: Research Excellence Award, Alliance Pharmaceutical Corporation. San Diego, CA.

1987-1988: Scientist Exchange Award (Postdoctoral Fellowship), French Cancer Society, Paris, France.

1984-1987: Graduate Student Scholarship, French Cancer Society, Paris, France.

PROFESSIONAL SERVICE

2007: Chair, Block symposium, regulation of immune cell development and function, American Association of Immunologis, Miami, FL.

2006-2010: Panel member, Hypersensitivity, Autoimmune and Immune-mediated Diseases (HAI) study section.

2006: Chair, Block symposium, treatment of autoimmune disease, American Association of Immunologis, Boston, MA.

2006: Review panel member, research proposals on Neurosciences, La Marato de TV3 Foundation, Catalan Agency For Health Technology Assessment And Research

2005: Chair, Block symposium, Cytokines and autoimmunity, American Association of Immunologis, Experimental Biology Meeting, San Diego, CA.

2004: Panel member, NIAID Biodefence Workshop, Immunization and Vacination in Special Populations, Division of Allergy, Immunology and transplantation, NIH, Bethesda, MD

2004:	Chair, Block symposium, Tolerance and regulation of autoimmunity, American Association of Immunologis, Experimental Biology Meeting, Washington DC.
2004-present:	Adhoc Reviewer, TTT Study section, National Institutes of Health
2004-present:	Adhoc Reviewer, HAI Study section, National Institutes of Health
2003	Adhoc Reviewer, IMS Study Section, National Institutes of Health
2003	Adhoc Reviewer, ALY Study Section, National Institutes of Health
2003-present:	Member, Molecular Biology Program, University of Missouri-Columbia
2003-present:	Member, Genetics Area Program, University of Missouri-Columbia
2003-present:	Member, Veterinary Pathobiology Area Program, University of Missouri-Columbia
2003-present	Scientific Consultant, Division of endocrinology and Diabetes, Unversity of Missouri, Kansas City, MO
2002-2004:	Scientific Consultant, Alliance Pharmaceutical, San Diego, CA.
2001-present:	Member of The Graduate Student Recruitment Committee, Department of Molecular Microbiology and Immunology, The University of Missouri School of Medicine, Columbia.
2000-2001:	Adhoc Reviewer, BM-1 Study Section, National Institutes of Health
1992-2000:	Editorial board member: *Viral Immunology*
1989-present:	Reviewer: Immunology Journals
2000:	Guest Editor, International Review of Immunology
2000-2001:	Chair, Graduate Student Advisory Committee, Genome, Science, and Technology program, Oak Ridge National Laboratories and The University of Tennessee, Knoxville.
1995-2001:	Member of The Graduate Student Recruitment Committee, Department of Microbiology, The University of Tennessee, Knoxville.
1998:	Member of Faculty Search Committee, Department of Comparative Medicine, College of Veterinary Medicine, The University of Tennesee, Knoxville.
1999:	Panel Member: NIH/NCI, Small Business Innovation Research (SBIR)/Small Business Technology Transfer (STTR) Grant program.

Flexible system to advance innovative research for cancer drug discovery by small business panel.

PROFESSIONAL MEMBERSHIP

2006-present: Member of the Henry Kunkel Society
1998-present: Member of the Society for Neuroscience
1992-present: Member of the American Association for the Advancement of Science.
1992-present: Member of the American Association of Immunologists.

PUBLICATIONS

Manuscripts published in peer-review journals

1. Bot, A., D. Smith, B. Phillips, S. Bot, C. Bona, and H. Zaghouani. (2006). Immunologic control of tumors by *in vivo* FcgR-targeted antigen loading in conjunction with dsRNA-mediated immune modulation. *J. Immunol.* 176:1363-1374.

2. 58. Caprio-Young, J., J. J. Bell, H-H. Lee, J. S. Ellis, D. M. Nast, G. Sayler, B. Min, and H. Zaghouani. (2006). Neonatally Primed Lymph Node but not Splenic T Cells Display a Gly- Gly Motif Within the T Cell Receptor Beta Chain Complementarity Determining Region 3 (CDR3) That Controls Affinity and Lymphoid Organ Retention. *J. Immunol.* 176:357-364.

3. Yu, P., R. K. Gregg, J. J. Bell, J. S. Ellis, R. Divekar, H-H Lee, R. Jain, H. Waldner, J. C. Hardaway, M. Collins, V. K. Kuchroo, and H. Zaghouani. (2005). Specific T regulatory cells (Tregs) display broad suppressive functions against experimental allergic encephalomyelitis upon activation with cognate antigen. *J. Immunol.* 174:6772-6780.

4. Gregg, R. K., J. J. Bell, H-H. Lee, R. Jain, S. J. Schoenleber, R. Divekar, and H. Zaghouani (2005). IL-10 diminishes CTLA-4 expression on islet-resident T cells and sustains their activation rather than tolerance. *J. Immunol.* 174: 662-670.

5. Gregg, R. K., R. Jain, S. J. Schoenleber, R. Divekar, J. J. Bell, H-H. Lee, P. Yu, and H. Zaghouani. (2004). A sudden decline in active membrane-bound TGFβ impairs both T regulatory cell function and protection against autoimmune diabetes . *J. Immunol.* 173:7308-7316.

6. Li, L, H-H. Lee, J. J. Bell, R. K. Gregg, J. S. Ellis, A. Gessner, and H. Zaghouani. (2004). IL-4 Utilizes an Alternative Receptor to Drive Apoptosis of Th1 Cells and Skews Neonatal Immunity Towards Th2. *Immunity.* 20: 429-440.

7. Bell, J. J., B. Min, R. Gregg, H-H. Lee, and H. Zaghouani. (2003). Break of neonatal Th1 tolerance and exacerbation of experimental allergic encephalomyelitis by interference with B7 costimulation. *J. Immunol.* 171:1801-1808.

8. Legge, K. L., Gregg, R. K. Maldonado-Lopez, R., Li, L., Caprio, J. C., Moser, M., and Zaghouani, H. (2002). On the role of dendritic cells in peripheral T cell tolerance and modulation of autoimmunity. J. Exp. Med. 196:217-227.

9. Pack, C. D., Cestra, A. E., Min, B., Legge, K. L., Li, L., Caprio, J. C., Bell, J. J., Gregg, R. K., and Zaghouani, H. (2001). Neonatal exposure to antigen primes the immune system to develop responses in various lymphoid organs and promotes bystander regulation of diverse T cell specificities. *J. Immunol.* 167:4187-4195.

10. Li, L., Legge, K. L, Min, B., Bell, J. J, Gregg, R., Caprio, J. and Zaghouani, H. (2001). Neonatal immunity develops in a transgenic TCR transfer model and reveals a requirement for elevated cell input to achieve organ-specific responses. *J. Immunol.* 167:2585-2594

11. Min, B., Legge, K. L., Li, L., Caprio, J. C., Gregg, R. K., Bell, J. J., and Zaghouani, H. (2001). Defective expression of CD40L undermines both IL-12 production by antigen presenting cells and up-regulation of IL-2 receptor on splenic T cells and perpetuates INFγ-dependent T cell anergy. *J. Immunol.* 166:5594-5603.

12. Day, R. B., Okada, M., Ito, Y., Tsukada, K., Zaghouani, H., Shibuya, N., and Stacey, G. (2001). Binding site of chitin oligosaccharides in the soybean plasma membrane. *Plant. Phys.* 126:1-12.

13. Legge, K. L., Min, B., Caprio, J. C., Li, L., Gregg, R. K., Bell, J. J., and Zaghouani, H. (2000). Coupling of peripheral tolerance to endogenous IL-10 promotes effective modulation of myelin-activated T cells and ameliorates experimental allergic encephalomyelitis. *J.Exp.Med.* 191:2039-51.

14. Anderson, A. C., Nicholson, L. B., Legge, K. L., Turchin, V., Zaghouani, H., and Kuchroo, V. K. (2000). High frequency of auto-reactive myelin proteolipid protein (PLP)-specific T cells in the periphery of naïve mice: mechanisms of selection of the self-reactive repertiore. *J. Exp. Med.* 191:761-770.

15. Min, B., Legge, K. L., Caprio, J. C., Li, L., Gregg, R., and Zaghouani, H. (2000). Differential control of neonatal tolerance by antigen dose versus extended exposure and adjuvant. *Cell. Immunol.* 200 :45-55.

16. Legge, K. L., Min, B., Pack, C. D., Caprio, J. C., and Zaghouani, H. (1999). Differential presentation of an altered peptide within fetal central and peripheral organs supports an avidity model for thymic T cell development and implies a peripheral re-adjustment for activation. *J. Immunol.* 162:5738-46.

17. Min, B., Legge, K. L., Pack, C. D. and Zaghouani, H. (1998). Neonatal exposure to a self peptide-Ig chimera circumvents the use of adjuvant and confers resistance to autoimmune disease by a novel mechanism involving IL-4 lymph node deviation and INFγ-mediated splenic anergy. *J. Exp. Med.* 188:2007-17.

18. Legge, K. L., Min, B., Cestra, A.E., Pack, C. D., and Zaghouani, H. (1998). T cell receptor agonist and antagonist exert in vivo cross-regulation when presented on immunoglobulins. J. Immunol. 161:106-11.

19. Legge, K. L., Min, B., Potter, N.T., and Zaghouani, H. (1997). Presentation of a T cell receptor antagonist peptide by immunoglobulins ablates activation of T cells by a synthetic peptide or protein requiring endocytic processing. *J. Exp. Med.* 185:1043-53.

20. Brumeanu, T-D, Dehazya, P., Wolf, I., Bot, A., Bona, C., and Zaghouani, H. (1996). Engineering of double antigenized Igs carrying B and T cell epitopes. Immunotechnology 2:85-95.

21. Brumeanu, T-D., Zaghouani, H., and Bona, C. (1995). Purification of antigenized immunoglobulins derivatized with monomethoxypolyethylene glycol. *J. Chromatogr.* 696:219-25.

22. Brumeanu, T-D., Zaghouani, H., Elahi, I., Daian, C. and Bona, C. (1995). Derivatization with monomethoxypolyethylene glycol of Igs expressing viral epitopes obviates adjuvant requirement. *J. Immunol.* 154:3088-95.

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APPENDIX B

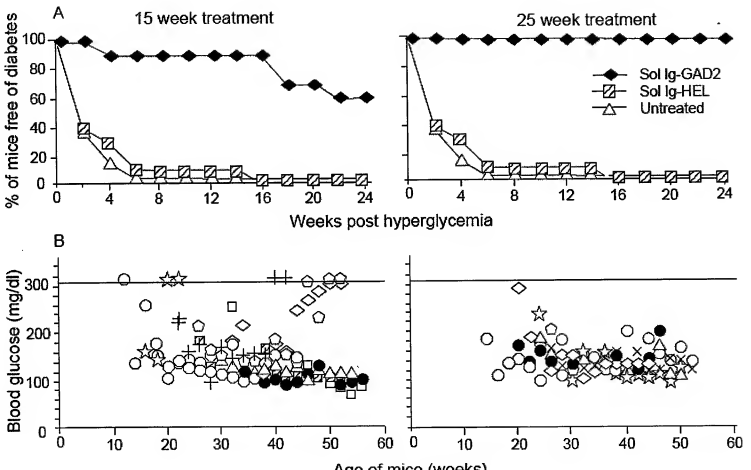


Figure 1

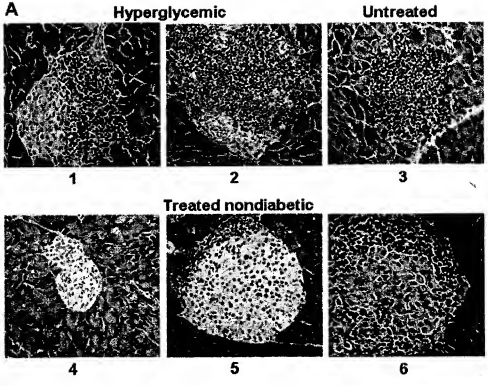


Figure 2

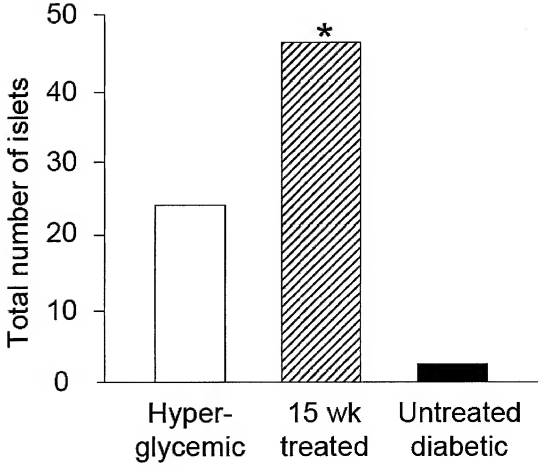

Total number of islets
50
40
30
20
10
0
*
Hyper-
glycemic
15 wk
treated
Untreated
diabetic

Figure 3

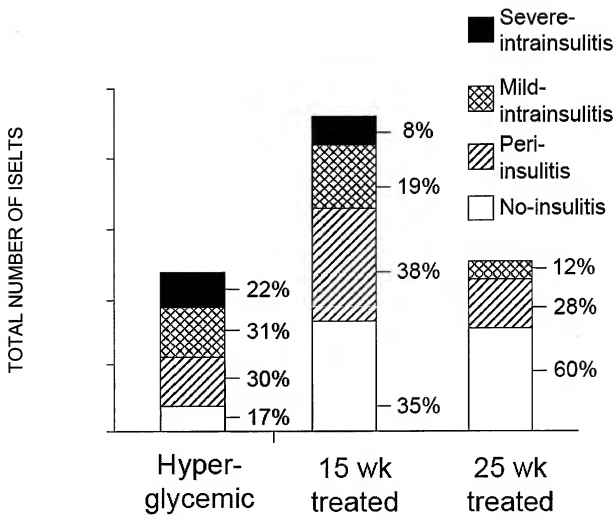

TOTAL NUMBER OF ISELTS
Severe-intrainsulitis
Mild-intrainsulitis
Peri-insulitis
No-insulitis
22%
31%
30%
17%
8%
19%
38%
35%
12%
28%
60%
Hyper-glycemic
15 wk treated
25 wk treated

Figure 4

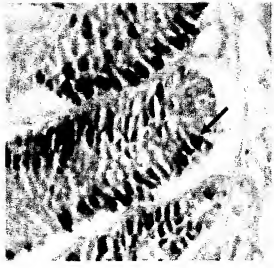
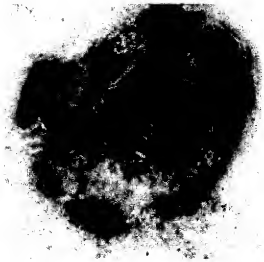


Figure 5



Normal

Figure 6

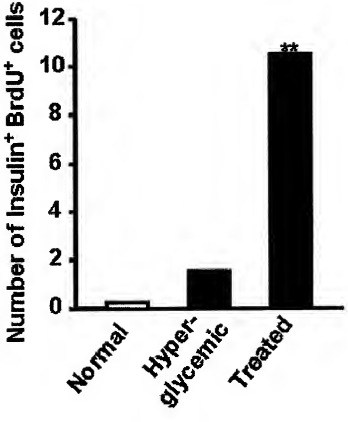

Number of Insulin+ BrdU+ cells
12
10
8
6
4
2
0
**
Normal
Hyper-glycemic
Treated

Figure 7

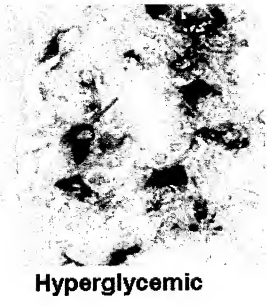
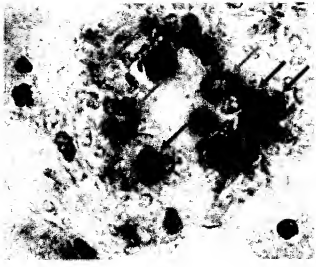


Figure 8



Treated

Figure 9

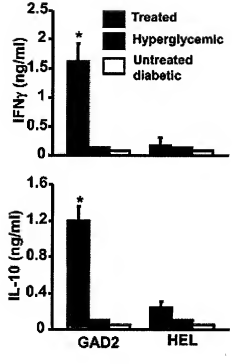

2.5
2
1.5
1
0.5
0
IFNγ (ng/ml)
*
Treated
Hyperglycemic
Untreated diabetic
1.6
1.2
0.8
0.4
0
IL-10 (ng/ml)
*
GAD2
HEL

Figure 10

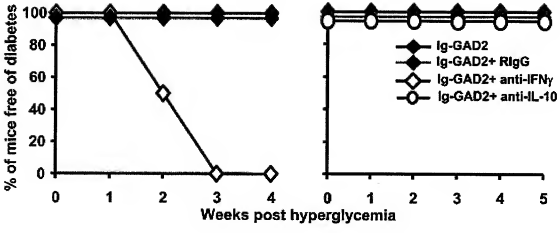

% of mice free of diabetes
100
80
60
40
20
0
0
1
2
3
4
0
1
2
3
4
5
Weeks post hyperglycemia
Ig-GAD2
Ig-GAD2+ RIgG
Ig-GAD2+ anti-IFNγ
Ig-GAD2+ anti-IL-10

Figure 11